Pest Management Grants <u>Final</u> Report

Development of Alternative Strategies for Chemical Control of Pre- and Postharvest Brown Rot of Stone Fruits

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Table 1.   Number of orchards detected for latent infection of stone fruits by *Monilinia fructicola* using overnight freezing technique (ONFIT) [x] [y]

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Abstract

An <u>O</u>ver<u>N</u>ight <u>F</u>reezing – Incubation <u>T</u>echnique (ONFIT) has been developed that can easily detect latent infection by *Monilinia fructicola*, the pathogen causing brown rot of stone fruit. Latent infection was detected in 24 of 29 orchards and preharvest brown rot in 25 orchards. Latent infection levels determined monthly are being compared to preharvest brown rot and to postharvest brown rot to determine the relative importance of short- and long-term latent infection in the epidemiology of fruit brown rot. Development of a nucleic acid-based system (PCR) for detection of latent infection is in progress.

Thinned fruit can provide spore inoculum of *M. fructicola* for secondary infections. Two passes with a rototiller over the orchard floor buried 95% of the thinned fruit spread on the ground. A cover spray of iprodione (Rovral®) at 1-pound a.i. in 50 gallons of water per acre suppressed sporulation and spore germination of *M. fructicola* on thinned fruit in two of four orchards.

Several *Trichoderma* isolates and yeasts tested as biocontrol agents reduced brown rot on peaches and plums when fruit were inoculated with *M. fructicola* following the application of a biocontrol agent. Some of the isolates controlled brown rot even under drier (50% relative humidity) conditions.

Executive Summary

The objectives of this work were a) To determine the relative importance of long- and short-term latent infection for pre- and post-harvest brown rot of stone fruits, b) To epidemiologically and economically evaluate alternative strategies for chemical control of pre- and post-harvest brown rot, and c) To develop biological control agents for postharvest treatment of brown rot.

An OverNight Freezing – Incubation Technique (ONFIT) has been developed which detects latent infection in stone fruit. Samples of fruit without symptoms were collected from 29 stone fruit orchards located in the Central Valley during May to August. The samples were divided in two lots, one used for the ONFIT and the second for a molecular detection method for latent infection using a polymerase chain reaction (PCR) approach. Briefly, the ONFIT technique consists of four major steps: (a) surface sterilizing fruit in a solution of 160 ml of chlorine (400 ppm) plus 160 ml ethanol and 0.5 ml Tween-20 in 10 liters water for 4 minutes; (b) placing sterilized fruit on galvanized wire screens in plastic containers and freezing them at -6°C for 12 to 15 hours, then allowing to thaw; (c) incubating thawed fruit at 23°C for 5 to 7 days; and (d) recording the incidence of brown rot on fruit by examining for sporulation of *M. fructicola*. Incidence of brown rot was higher in the fruit stem end than the equator and the apex (and stylar end), suggesting that a major portion of stone fruit are infected by direct *Monilinia* contamination of the wound that is made when a fruit is picked by separating it from its pedicel.

PCR-based detection system is being developed. Because of difficulties in amplifying DNA of *M. fructicola* by using previously reported primers, we designed our own primers. We started to run random amplified polymorphic DNA (RAPD) with Operon random primers to find specific bands in the fingerprint of *M. fructicola*. We designed two pairs of primers accordingly and tested these primer with fungal genomic DNA of *M. fructicola* and other species such as *M. laxa*, *Botrytis cinerea, Alternaria alternata,* and other fungi. We also extracted DNA of apparently healthy and inoculated stone fruit using both Promega Wizard Genomic DNA Extraction Kit and modified Dellaporta Miniprep method. Serial dilution of total DNA of healthy and inoculated fruit was then used to test specific primers we designed. Development of a nucleic acid-based system (PCR) for detection of the latent infection is in progress.

Pre-harvest brown rot was assessed in each of the 29 orchards from where samples of immature fruit were taken. Post-harvest brown rot was also determined subsequently by harvesting and incubating fruit at 20º C for several days. In 1998, there were no significant correlations of the latent infections (detected by the ONFIT) and the incidence of pre- and post-harvest fruit brown rot in 1998. However, in previous years' research, significant correlations between latent infections and pre- and post-harvest fruit brown rot were detected in peaches, nectarines, plums, and prunes.

To investigate the second objective, sporulation of *M. fructicola* was examined in pre-pit-hardening thinned fruit in 3 commercial nectarine orchards. In addition, thinned fruit were buried underneath by rototilling and compared with thinned fruit left intact on the orchard floor. In a third experiment in four orchards, thinned fruit on the ground were sprayed with iprodione (Rovral®; 1 pound a.i. in 50 gallons of water per acre) in replicated plots and compared with thinned fruit that were not sprayed. The results indicated that there was no sporulation of *M. fructicola* in pre-pit-hardening fruit but comparison with sporulation on post-pit-hardening fruit was not made since growers do not practice post-pit-hardening thinning any longer. Sporulation of *Monilinia fructicola* on thinned fruit in 1998 was very low and detected in only 1 of 3000 thinned fruit in three orchards. Two passes with a rototiller over the orchard floor buried 95% of the thinned fruit spread on the ground. Spraying thinned fruit with iprodione reduced the incidence and density of sporulation and spore germination of *M. fructicola* on thinned fruit in 2 out of 4 orchards where these experiments were performed. Pre- and postharvest brown rot, however, was low even in the two orchards where sporulation on thinned fruit was observed, which might have been due to frequent application of protective sprays of registered fungicides by the grower.

Several aspects of biological control of *M. fructicola* were studied. First, we collected a large number of biocontrol agents from fruit, leaves, mummified and thinned fruit, and other microflora of stone fruits. Second, we screened these antagonists for efficacy against *M. fructicola.* And third, we studied the effect of timing and concentration of the biological control agent against brown rot on Casselman plums. All the *Trichoderma* isolates tested reduced significantly the incidence of brown rot in both peaches and plums. Some of the yeast isolates also reduced brown rot incidence significantly. The best control on plums was achieved when the biocontrol agent was applied 12 hours earlier than the inoculation with spores of *M. fructicola*, suggesting that some of these biocontrol agents can act as protectants against infection by the brown rot fungus. Two biocontrol isolates, Ta291 and 23-E-6, reduced brown rot significantly under even drier incubation conditions (50% RH).

Generally speaking, we have shown that alternatives to chemical control of brown rot of stone fruits exist can be developed but more research is needed to improve these approaches and at the same time develop even more alternatives for disease control.

Introduction

California has 100 000 hectares of stone fruit industry, yearly producing 1.4 million tons of fruits, worth an estimated $649 million (U.C. Fruit & Nut Research and Information Center, 1996). California's stone fruit industry is also nationally and socially of great importance, because in the United States, 87% to nearly 100% of various stone fruits are produced in California. Unfortunately, this industry suffers tremendous losses due to brown rot, a most destructive disease caused mainly by *Monilinia fructicola* (Wint.) Honey. Despite extensive application of fungicides, average yield loss caused by this disease is about 10 to 15%. During wet years, it was not uncommon that brown rot destroyed the majority of the fruit in some orchards (15,16).

This disease usually starts as a blossom blight in the spring, then develops into a twig blight and spreads to cause latent infection of green fruit; subsequently, brown rot develops on fruit at the end of the season and after harvest (during storage and transit to market). Pre- and post-harvest fruit rot is the most important phase of this disease epidemiologically and economically (17-19).

Latent infection is an important stage of fruit brown rot development in stone fruit. There are two categories of latent infection according to the fruit developmental stages when the infections take place and their inoculum sources. Long-term latent infections take place at an early stage of fruit development and result mainly from primary inoculum (7,9,11) or inoculum produced in primary infections (blighted blossoms, etc.), while short-term latent infections take place at a later stage of fruit development and result mainly from typically secondary inoculum produced on thinned fruit (1,10,13), non-abscised aborted fruit and infected injured green fruit (1,13).

It was demonstrated that short-term latent infections are of great significance in the management of brown rot of stone fruits in the Murrumbidgee Irrigation Areas in Australia (12). Reports from Canada (1) and South Carolina (13) also indicated that typically secondary inoculum plays an important role in fruit rot development and implicitly supported the significance of short-term latent infection. Our investigations provided quantitative data further demonstrating the significance of thinned fruit as a secondary inoculum source for brown rot in California irrigated nectarine orchards (10).

Current approaches for brown rot control emphasize chemical protection of the blossoms in spring followed by two to three cover sprays and one to two preharvest sprays and a postharvest treatment. Repeated application of fungicides has induced the resistance of the brown rot fungus to bezimidazole (benomyl) (14) and dicarboximide (iprodione) fungicides (4) and reduced the sensitivity to demethylation inhibitor (DMI) fungicides (triforine) (3). Also, choices of fungicides for brown rot control are very limited because of increasing public concern about pesticide residues on fruit and environmental pollution. There has been no registered

fungicide for postharvest treatment for control of stone fruit brown rot, since Rovral was canceled by the Registrar, Rhône-Poulenc Ag. Company in 1995. The industry is at great risk of suffering great losses. There is an urgent need to investigate alternatives to chemical control of this disease.

There are possible alternatives to chemical control of brown rot. One of the best strategies could be to target the inoculum sources and reduce the amount of inoculum such as conidia produced on thinned fruit. This strategy would lead to a substantial reduction in fungicide residues on fresh fruits and even in air and soil contamination without loss of disease control. In addition, biocontrol agents remain promising substitutes for chemical control of postharvest brown rot of stone fruits (5,7).

Materials and Methods

Objective 1: *To determine the relative importance of long- and short-term latent infection for pre- and post-harvest brown rot of stone fruits*

Symptomless fruit was sampled from 29 orchards in the Central Valley. Three samples of 40 fruit were collected from ten trees in each of these orchards periodically starting from May until August. Sampled fruit was divided into two lots, one each for detection of latent infection by the overnight freezing incubation technique (ONFIT) and polymerase chain reaction (PCR) based approach.

The ONFIT technique consists of four major steps: (a) surface sterilizing fruit by dipping them in a solution of 160 ml of chlorine (400 ppm) plus 160 ml ethanol and 0.5 ml Tween-20 in 10 liters water for 4 minutes; (b) placing sterilized fruit on galvanized wire screens in plastic containers and freeze them at -6°C for 12 to 15 hours, then allowing to thaw; (c) incubating thawed fruit at 23°C for 5 to 7 days; and (d) recording the incidence of brown rot infected fruit by examining for sporulation of *M. fructicola* in each of replicated samples. In addition, the site (part) of fruit exhibiting the sporulation was noted for each infected fruit.

PCR-based detection system is being developed. Primers *mfs-3 and NS5* were designated for amplifying DNA of *M. fructicola* as reported previously by Fulton and Brown (5). But we were unable to amplify the DNA of California isolates of this fungus with this pair of primers and original protocol. We also tried to re-optimize the protocol by changing volumes of PCR buffer, MgCl$_2$, dNTPs, DNA template, and increasing or decreasing annealing temperature with no success. Consequently, we had to design our own primers. We started to run random amplified polymorphic DNA (RAPD) with Operon random primers to find specific bands in the fingerprint of *M. fructicola*. The DNA fragment of specific bands was subsequently purified with Qiagen QIAquick Gel Extraction Kit, ligated and transformed using Invitrogen TOPO^{TM} TA Cloning Kit. The plasmid DNA was extracted using QIAPrep Spin Miniprep Kit, digested using enzyme Ecor I, and examined through electrophoresis before sent for sequencing. We designed

two pairs of primers accordingly and tested these primer with fungal genomic DNA of *M. fructicola* and other species such as *M. laxa*, *Botrytis cinerea, Alternaria alternata,* and so on. We also extracted DNA of apparently healthy and inoculated stone fruit using both Promega Wizard Genomic DNA Extraction Kit and modified Dellaporta Miniprep protocol (2). Serial dilution of total DNA of healthy and inoculated fruit was then used to test specific primers we designed.

Preharvest brown rot was assessed in each of these orchards by counting the number of diseased fruit exhibiting sporulation of *M. fructicola* out of 200 fruit in each of 10 randomly selected trees, 1 or 2 days before the commercial harvest started. In addition, on the same day, one box of sound fruit (25 fruit/box) was harvested from each of the ten trees, stored at 1°C for a week to suppress Rhizopus rot, then incubated at 20°C and high relative humidity (95%) for 3 to 5 days to induce brown rot development. Postharvest brown rot was determined subsequently by counting the brown rot fruit in each box. Statistical analysis will be conducted to determine the correlation between the long- and short-term latent infections of different sampling dates and pre- and post-harvest brown rot.

Objective 2: *To epidemiologically and economically evaluate alternative strategies for chemical control of pre- and post-harvest brown rot of stone fruits.*
The strategies evaluated included (1) thinning of excess fruit at the pre-pit-hardening; (2) burying thinned fruit underneath by disking or rototilling (2a), and/or cover spraying thinned fruit on the floor with chemicals (2b) (vs. conventional practice, leave thinned fruit on the floor).

Since conidia of brown rot fungus are airborne, the size of plot was placed at the top priority when planning field trial. Only one strategy [1] or sub-strategy [2a & 2b] was evaluated in comparison with the conventional practice in any orchard to maximize the plot size. Three pairs of comparisons (thinning at the pre- vs. post-pit-hardening, burying thinned fruit by disking vs. conventional, and cover spraying thinned fruit vs. conventional) was carried out in six orchards, with two orchards each pair). In each orchard, there were three replicated plots per treatment and plots were arranged in a randomized block design.

Pre- vs. post-pit-hardening thinning. One hundred pre-pit-hardening thinned fruit under each of 10 trees were examined for sporulation of *Monilinia fructicola* in three commercial nectarine orchards, respectively.

Burying thinned fruit underneath by disking or rototilling vs. conventional practice, leave thinned fruit on the floor. Five boxes, 400 thinned fruit/box were collected from two commercial nectarine (cvs. August Red and September Red) orchards, Reedley, and spread on the ground with one box of fruit under each tree in an experimental orchard at Kearney Agricultural Center. The thinned fruit were buried through two passes of the rototiller over the orchard floor

Cover-spray vs. conventional practice. This strategy was tested in one nectarine (cv. May Diamond) and one peach (cv. Spring Lady) orchard in Parlier, and two nectarine (cvs. August Red and September Red) orchards in Reedley. Excess green fruit was thinned at pit-hardening or post pit-hardening stage. The entire orchards received normal foliar sprays, which consisted of 3 to 4 applications. Both treated and untreated control were repeated 3 to 4 times in each of these

orchards with 121 to 128 trees/plot. Rovral was sprayed at 1-pound a.i. with 50 gallon of water per acre onto the thinned fruit in each of the treated plots in all orchards 7 to 11 days after thinning using a herbicide sprayer. A second spray of the same fungicide was followed in Reedley orchards 2 weeks later. Thinned fruit in treated and conventional plots was examined for sporulation of brown rot fungus and sampled for conidial quantification in the center trees accordingly. Also pre- and postharvest brown rot was determined as described previously.

Items and costs of conventional practice in terms of disease control after fruit thinning and extra labor or facility usage involved with experimental treatments was noted for each orchard.

Statistical analyses were carried out to determine the effects of alternative strategies on pre- and post-harvest brown rot of stone fruit and their benefit/cost.

Objective 3: *To develop biological control agents for postharvest treatment of brown rot.*

Twenty isolates of *Trichoderma* spp. and 4 isolates of yeast were tested with a variety of fruits (peach, nectarine, and plum). Some biological agents were also applied 12 h earlier, at the same time, or 12 h later than inoculation of *M. fructicola* to determine the effect of timing on biological control of this disease. In addition, the effect of dosage of three *Trichoderma* isolates on biocontrol was studied.

Results and Discussion

Objective 1: *To determine the relative importance of long- and short-term latent infection for pre- and post-harvest brown rot of stone fruits to help target significant sources of inoculum*

(a) Detection of latent infection by overnight freezing technique (ONFIT). Latent infection was detected in fruit from the 24 orchards (Table 1). Preharvest brown rot was observed in 25 sampled orchards (Table 1). More latent infection was observed on the stem end than the equator and the apex of nectarines (Fig. 1). Postharvest brown rot is being assessed. Monthly data of latent infection are being related to preharvest brown rot and will be further to postharvest brown rot to determine the relative importance of long- and short-term latent infection.

(b) Development of a nucleic acid-based system for detection of the latent infection. Two pairs of primers Mf1A and Mf1B, Mf2A and Mf2B which we designed amplified DNA of *M. fructicola,* but not any other species we tested including *M. laxa, Botrytis cinerea,* many species of *Alternaria* and *Ucladium* (Fig. 2). Comparatively, annealing temperatures were 50°C for Mf1A and Mf1B and 55°C for Mf2A and Mf2B. Mf2A and Mf2B amplified DNA of all *M. fructicola* isolates and Mf1A and Mf1B amplified most of the isolates tested. Mf2A and Mf2B are very sensitive and detected and amplified fungal genomic DNA down to 1 pg/μl at 30 cycles (Fig. 3). Specificity and sensitivity of these primers is being tested with total DNA of apparently healthy and inoculated fruit.

Objective 2: *To epidemiologically and economically evaluate alternative strategies for chemical control of pre- and post-harvest brown rot of stone fruits*

(a) Thinning of excess fruit at the pre-pit-hardening vs. thinning at the post-pit-hardening. Sporulation was observed only on one out of 3000 thinned fruit examined. No comparison with post-pit-hardening thinning was made because of few stone fruit growers of currently practicing pre-pit-hardening thinning in the San Joaquin Valley and their small orchards.

(b) Burying thinned fruit underneath by disking or rototilling vs. conventional practice, leave thinned fruit on the floor. Five boxes, 400 thinned fruit/box were collected from two commercial nectarine (cvs. August Red and September Red) orchards, Reedley, and spread on the ground with one box of fruit under each tree in an experimental orchard at Kearney Agricultural Center. Following the two passes of the rototiller over the orchard floor, only 5% of the spread thinned fruit remained on the ground surface while 95% were buried underneath. No sporulation of *M. fructicola* was ever observed on any of the remaining fruit on the ground during the following 3 weeks.

(c) Cover spraying thinned fruit on the floor with chemicals. Sporulation was found on thinned fruit only in two Reedley orchards. Differences were subsequently observed in the incidence (Fig. 4) and intensity (Fig. 5) of the sporulation of *M. fructicola* on thinned fruit, and the germination (Fig. 6) of spores produced on thinned fruit between treated and untreated plots in both orchards on most of the sampling dates. However, no differences were observed in either preharvest or postharvest brown rot (Figs. 7&8). According to Fig. 5, a great quantity of 8 to 150×10^6 conidia per sporulating thinned fruit were detected. There should have been plenty of secondary inoculum for fruit brown rot in these two orchards. The low incidence of pre- and postharvest brown rot and lack of the difference between plots may have been due to frequent protective sprays of fungicides.

Objective 3: *To expand on the biological control of postharvest brown rot of stone fruits*

(a) Screening of biological agents for potential to control brown rot of stone fruits. The diameter of brown rot lesions was significantly (P=0.003) reduced on Elegant Lady peaches protected with BI-54 or Ta291 at either concentration of 10^7 and 10^8 spores/ml 4 days after inoculation (Fig. 9). Significant reduction of brown rot severity was achieved with Ta291 but not with BI-54 6 days postinoculation (Fig. 9). Reduction of brown rot severity was achieved on Fairtime peaches with four biological control agents only at the concentration of 10^8 spores/ml 5 days after inoculation (Fig. 9). Brown rot was observed 1, 2, and 6 days later on Casselman plums protected with BI-54 and 23-E-6, Ta291, and New than on control plums, respectively (Fig. 10). Brown rot was suppressed greatly (P<0.001) on plums protected with all biological control agents at both concentrations throughout the tests (11 days) except for BI-54 at 10^7 spores/ml (only 7 days) (Fig. 10).

Trichoderma isolates reduced the severity of peach brown rot by 63 to 98%; BI-54 reduced decay by 100% at concentration of 10^8 spores/ml and 87% at 10^7 spores/ml (Fig. 11). Isolates Ta291 and BI-54 gave discernibly better control of brown rot on peaches than the other isolates at 10^8 spores/ml (Fig. 11). However, the three *Trichoderma* isolates gave better control of brown rot on plums than the yeast isolate BI-54 at comparable concentrations (Fig. 11). The isolate New gave discernibly better control of brown rot than the other two *Trichoderma* isolates on plums. BI-54 reduced brown rot of plums only at 10^8 spores/ml but not at 10^7 spores/ml (Fig. 11).

(b) Timing of application of biological control agents against brown rot of plums. Four biocontrol agent isolates and two concentrations tested provided the best control of brown rot on plums when they were applied onto wounds 12 h earlier than the inoculation with *M. fructicola* (Fig. 12). The yeast isolate BI-54 suppressed brown rot only when it was applied 12 h earlier than the challenge inoculation and only in the first test but not in the second, a more critical test conducted under continuously high humidity condition. In contrast, at a concentration of 10^8 spores/ml the isolate New significantly suppressed brown rot even when it was applied onto the wounds 12 h later than the inoculation with *M. fructicola* in these tests Similarly, at a concentration of 10^8 spores/ml the other two *Trichoderma* isolates (Ta291 and 23-E-6) also suppressed brown rot substantially even when they were applied 12 h later than the inoculation with *M. fructicola* during the first test. Similar data were obtained from the third experiment (data not shown).

(c) Effect of concentration of biological control agent on brown rot of plums. Three *Trichoderma* isolates were tested for the effect of concentration of biocontrol agents on brown rot development. The incidence of brown rot decreased as the concentration of biological control agent (New or Ta291) increased (Fig.13). On plums satisfactory control was achieved with isolate New at 10^6 spores/ml, and with isolate Ta291 at 10^7 spores/ml, but not with isolate 23-E-6 at any concentrations tested.

Summary and Conclusions

Latent infection of stone fruits by *Monilinia fructicola* was detected using the overnight freezing technique (ONFIT) in 24 of 29 sampled orchards. Preharvest brown rot was observed in the 25 orchards. More brown rot was observed in the stem end of fruit than the equator and apex of these fruit. Postharvest brown rot is being assessed. Latent infection levels determined monthly are being compared to preharvest brown rot and eventually to postharvest brown rot to determine the relative importance of short- and long-term latent infection in the epidemiology of fruit brown rot. Development of a nucleic acid-based system for detection of the latent infection is underway.

Sporulation of *Monilinia fructicola* was observed only on 1 of 3000 thinned fruit in three orchards. Two passes of rototiller over the orchard floor buried 95% the thinned fruit spread on the ground. Cover spray of Rovral at 1-pound a.i. with 50 gallon of water per acre suppressed the sporulation (incidence and intensity) and spore germination of *M. fructicola* on thinned fruit in two of four orchards. No sporulation developed in the other two orchards. Pre- and postharvest brown rot, however, was low in the two orchards, which might have been due to frequent protective sprays of fungicides.

At concentrations of 10^7 and 10^8 spores/ml all *Trichoderma* isolates substantially reduced brown rot on peaches (63 to 98%) and plums (67 to 100%) when fruits were inoculated with *M. fructicola* following the application of a biological control agent. Similarly, at 10^8 spores/ml the yeast BI-54 also suppressed brown rot on peaches completely and on plums by 54%. Significant brown rot reduction was also achieved with the isolate New at a concentration of 10^8 spores/ml even when the biocontrol agent was applied 12 h after inoculation with *M. fructicola* and under continuous conditions of 95% relative humidity (RH). The isolates Ta291 and 23-E-6 also reduced brown rot significantly under drier (50% RH) incubation conditions. These isolates provided the best control of brown rot on plums when they were applied 12 h earlier than inoculation with *M. fructicola*. Satisfactory control of brown rot on plums inoculated with *M. fructicola* at 8×10^4 spores/ml was achieved with New at 10^6 spores/ml and with Ta291 at 10^7 spores/ml.

In conclusion, although we still do not have developed all the possible alternatives for the chemical control of brown rot, we at least were successful partially in developing some alternatives to brown rot and at the same time we are continuing our research to improve these approaches.

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List of Publication Produced

1. Hong, C.X., Michailides, T.J., and Holtz, B.A. Effects of wounding, inoculum density, and biological agents on postharvest brown rot of stone fruits. Plant Disease 82:1210-1216.

Table 1. Number of orchards detected for latent infection of stone fruits by *Monilinia fructicola* using overnight freezing technique (ONFIT) [x] [y]

Fruit	Sampled	Fruit with latent infection	Fruit with preharvest brown rot
Nectarine	4	2	3
Peach	8	6	8
Plum	5	5	4
Pluot	1	1	1
Prune	11	10	9 [z]

[x] Fruit surface-disinfested with 160 mL alcohol and 160 mL bleach plus 0.5 mL Triton 100 in 10 L water for 4 min, frozen at -14 for 12 to 14 h, then allowed to thawed and incubated at room temperature for 7 to 9 days.
[y] Postharvest brown rot is being assessed.
[z] Brown rot in one prune orchard was not determined because fruit picked.

Table 2. Biocontrol of postharvest brown rot of plums (cv. Casselman) by *Monilinia fructicola* at different timings of application of biological agents [y]

Biological agent	Concentration (spores/ml)	Control (%) at timings of application of biological agents		
		12 h earlier	Simultaneous	12 h later
New	10^8	100 a [z]	100 a	91 a
	10^7	100 a	100 a	19 bc
Ta291	10^8	93 a	98 a	36 b
	10^7	100 a	76 a	8 bc
23-E-6	10^8	100 a	69 a	-1 bc
	10^7	100 a	12 b	-12 bc
BI-54	10^8	1 b	1 b	-10 bc
	10^7	-3 b	-12 b	-19 c

[y] Treated fruit were incubated at 20°C and 95% relative humidity (RH) for 1 day, then incubated at 20°C and 50% RH for 6 days. The control of brown rot by biological agent was calculated as the difference of disease severity between untreated and treated fruit divided by the severity of untreated fruit.
[z] Numbers followed with the same letter within a column did not differ significantly according to Duncan multiple range test at *P*=0.01.
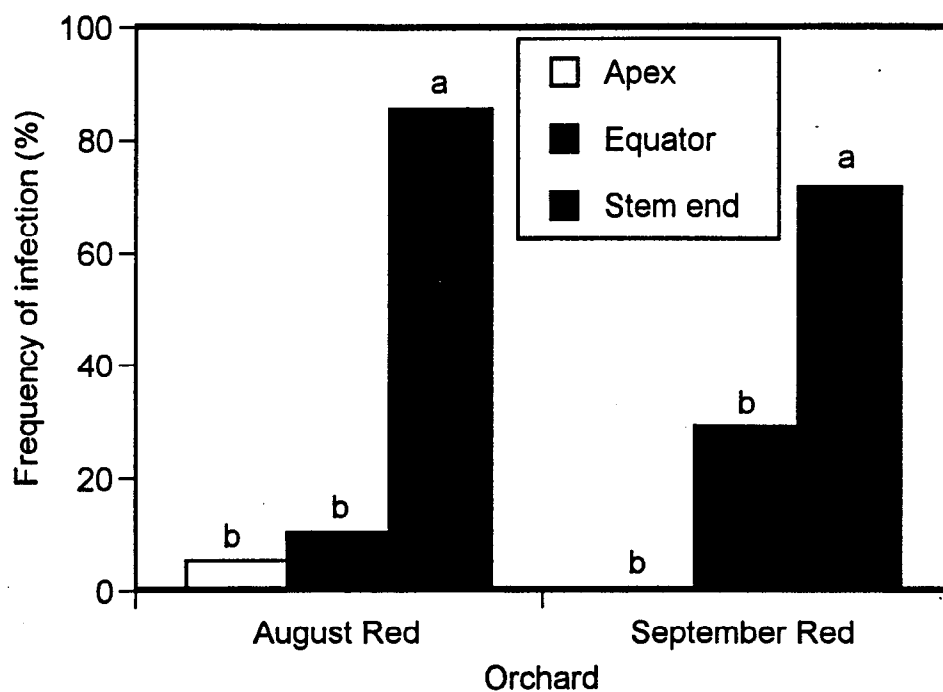
Figure 1. Relative frequency of infection by *Monilinia fructicola* on different parts of the nectarines from two commercial orchards, Reedley, 1998. Bars topped with different letters differed significantly at $P<0.01$.
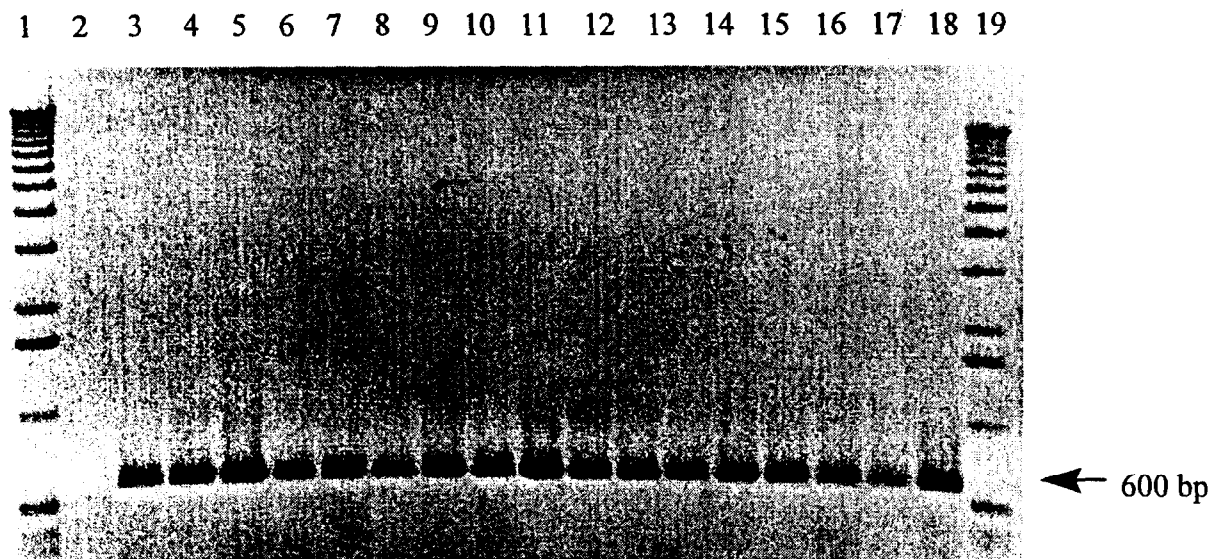
Figure 2. Gel electrophoresis of genomic DNA of *Monilinia fructicola* amplified by primers Mf2A and Mf2B. Lanes 1 and 19, DNA ladder; line 2, water control; and lanes 3 to 18, 16 different isolates of *M. fructicola*.
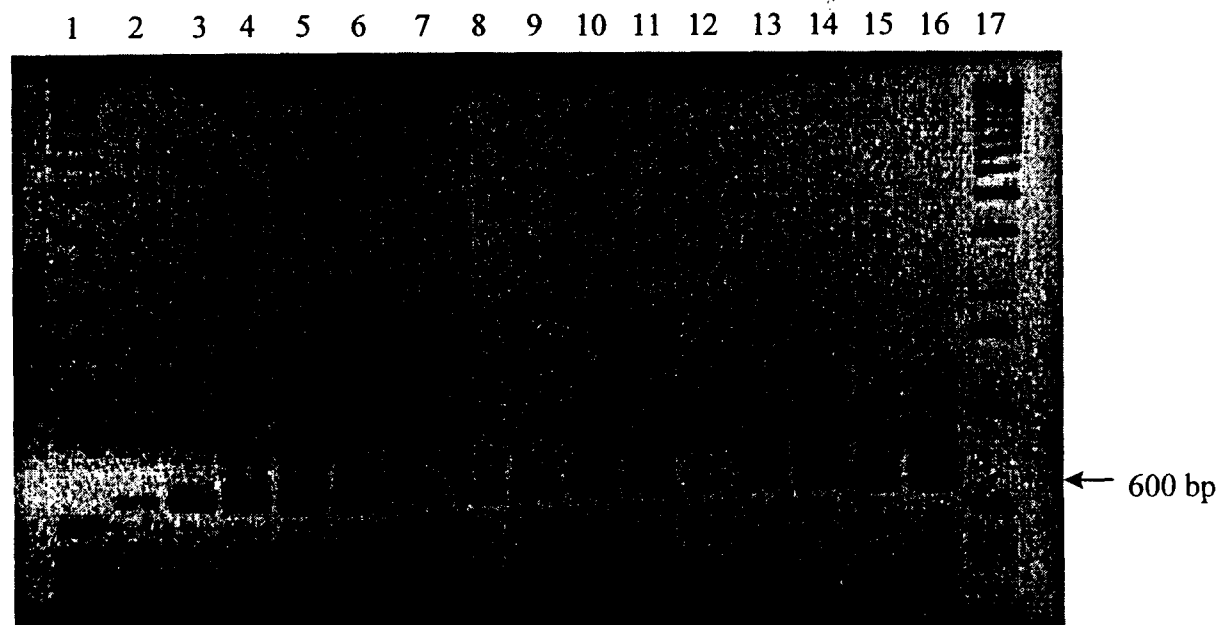
Figure 3. Gel electrophoresis of genomic DNA of three *Monilinia fructicola* isolates amplified by primers Mf2A and Mf2B with different amounts of template from 5, 50, 500, 5 000 to 50 000 pg. Lanes 1 and 17, DNA ladder; lanes 2 to 6, isolate Mf3; lanes 7 to 11, isolate Mf12; and lanes 12 to 16, isolate 33.
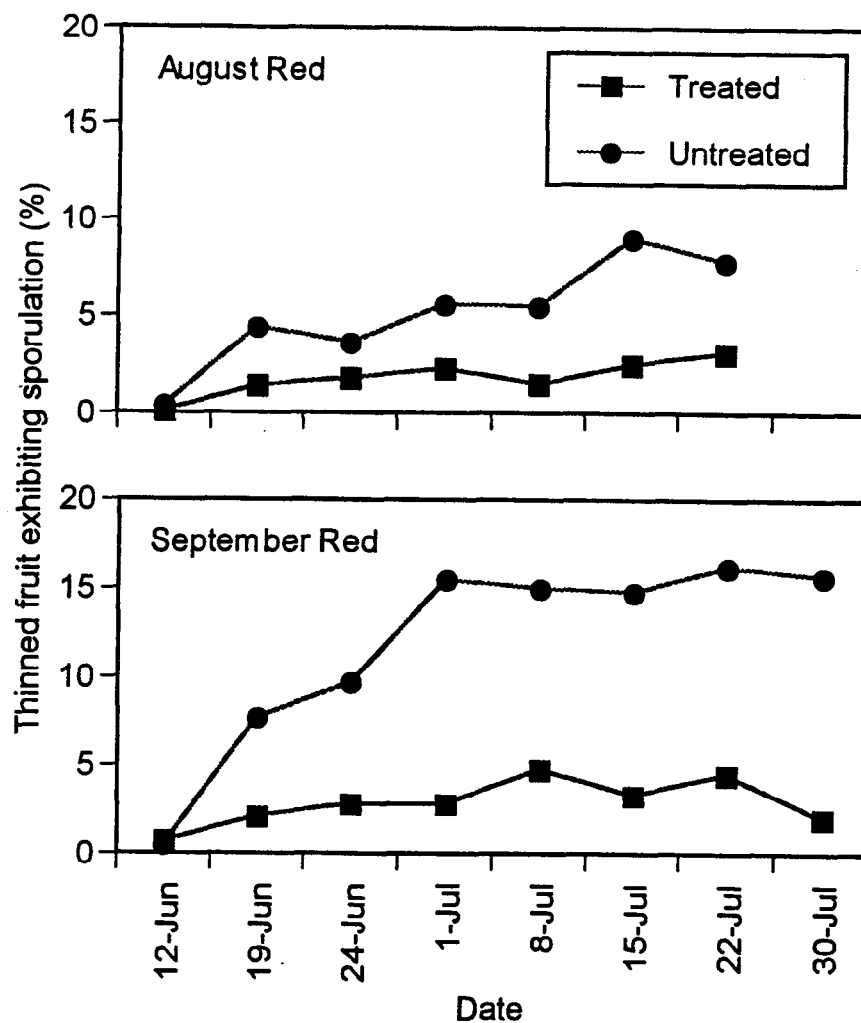
Figure 4. Thinned fruit exhibiting sporulation of *Monilinia fructicola* in two commercial nectarine orchards, Reedley, 1998. Fruit were thinned on June 1 to 3, and in treated plot they were cover-sprayed with Rovral at 1 pound a.i. in 50 gallon of water/acre on June 12 and 24, respectively
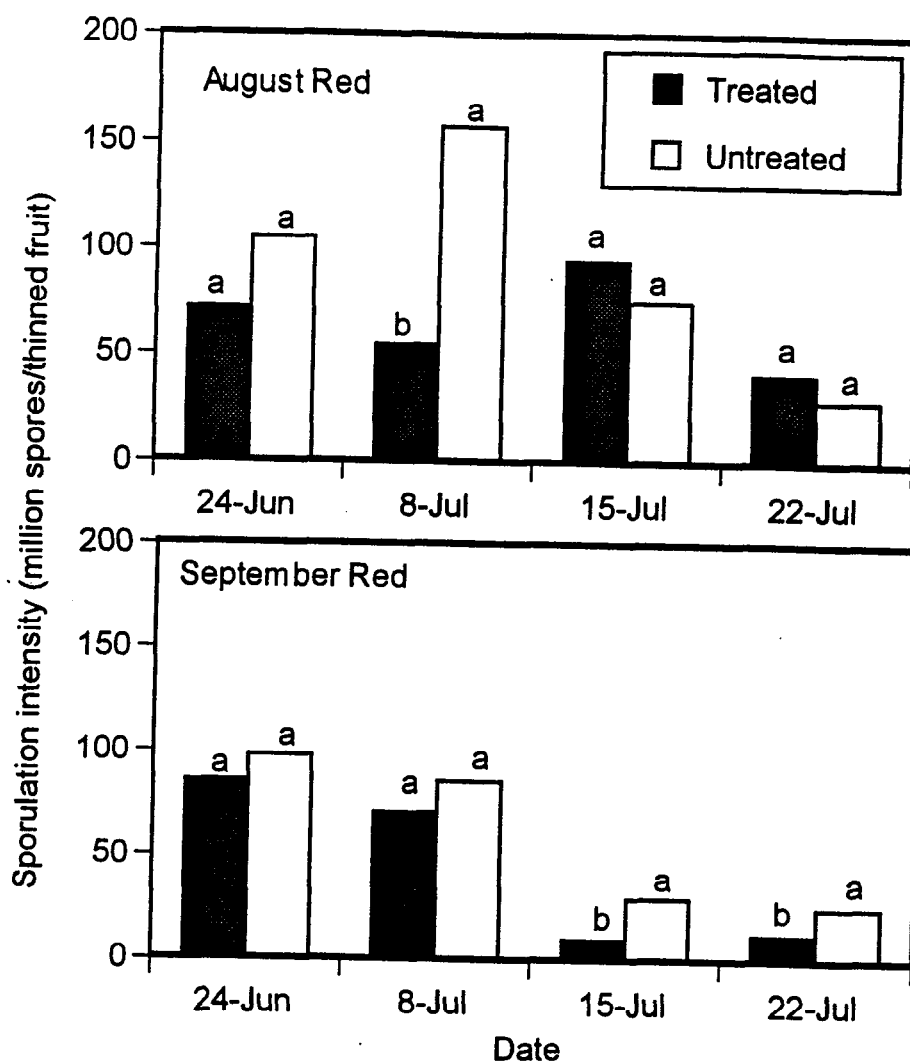
Figure 5. Sporulation intensity of *Monilinia fructicola* on fungicide-treated and -untreated thinned fruit in two commercial nectarine orchards, Reedley, 1998. The intensity was determined by sampling and washing five sporulating thinned fruit in 100 mL of sterile distilled water on a shaker for 30 min of each replicated plot. Bars topped with different letters of individual sampling dates and orchards differed significantly at $P<0.01$
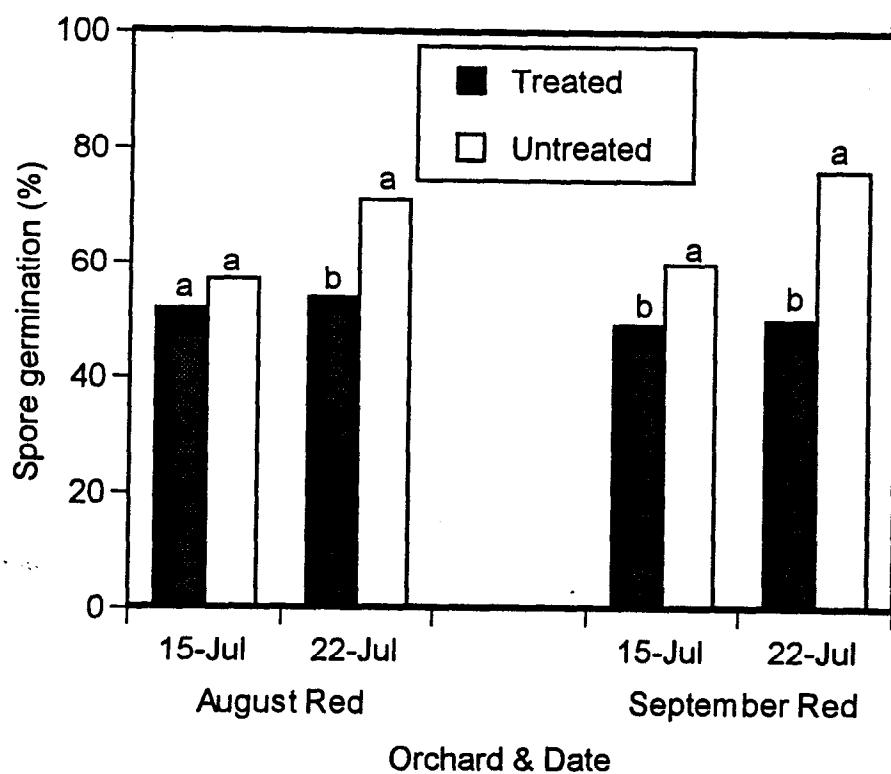
Figure 6. Germination of *Monilinia fructicola conidia* produced on thinned fruit in fungicide-treated and untreated plots of two commercial nectarine orchards, Reedley, 1998. The washings were examined for spore germination after 24 h incubation at 23 C and a spore was considered germinated when the germ tube is equal or longer than the width of spore. Bars topped with different letters of individual sampling dates and orchards differed significantly at $P<0.01$
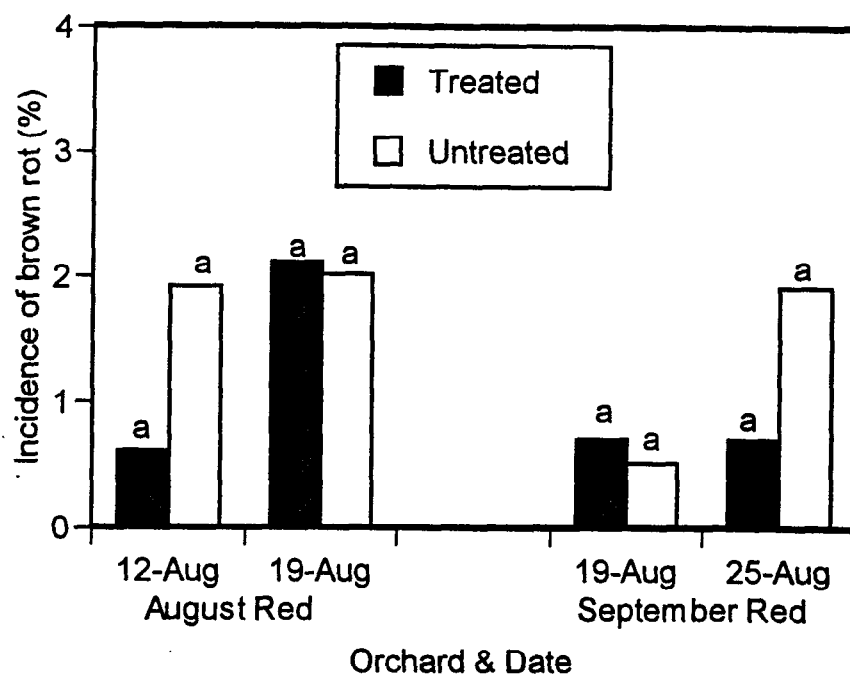
Figure 7. Preharvest brown rot of nectarines by *Monilinia fructicola* in thinned fruit-treated and -untreated plots of two commercial orchards, Reedley, 1998. One hundred fruit in each of four center tree of a plot were counted for sporulation. Bars topped with different letters of individual sampling dates and orchards differed significantly at *P*<0.05
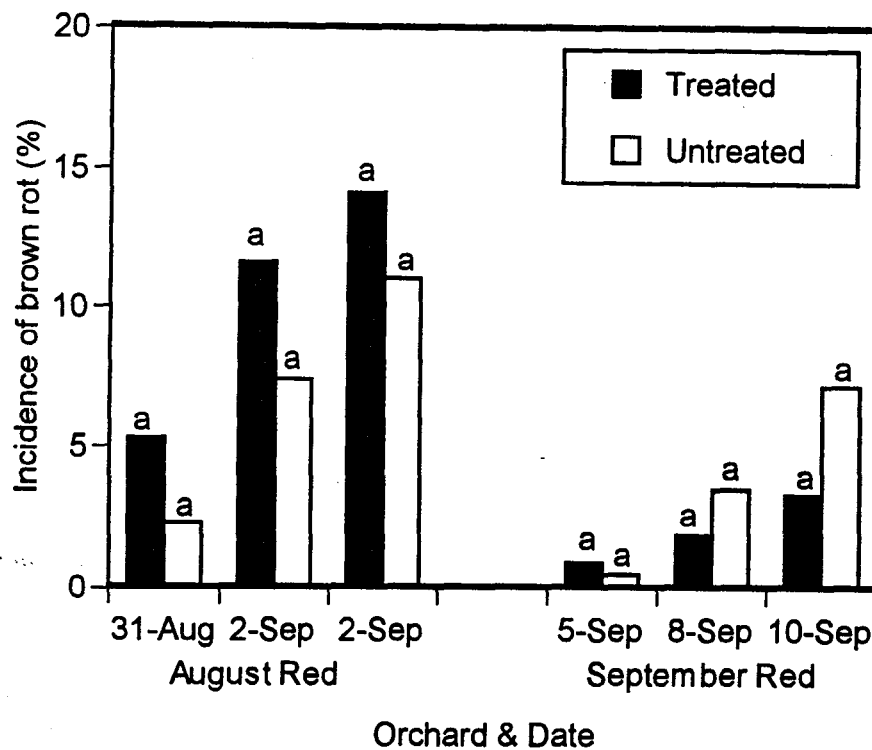
Figure 8.   Postharvest brown rot by *Monilinia fructicola* of nectarines from thinned fruit-treated and -untreated plots of two commercial orchards, Reedley, 1998. Fruit were first examined for brown rot after stored in cold room at 0.5 C for 7 days and incubated at 20 C and 95% raltive humidity for 3 days, Bars topped with different letters of individual examining dates and orchards differed significantly at $P<0.05$
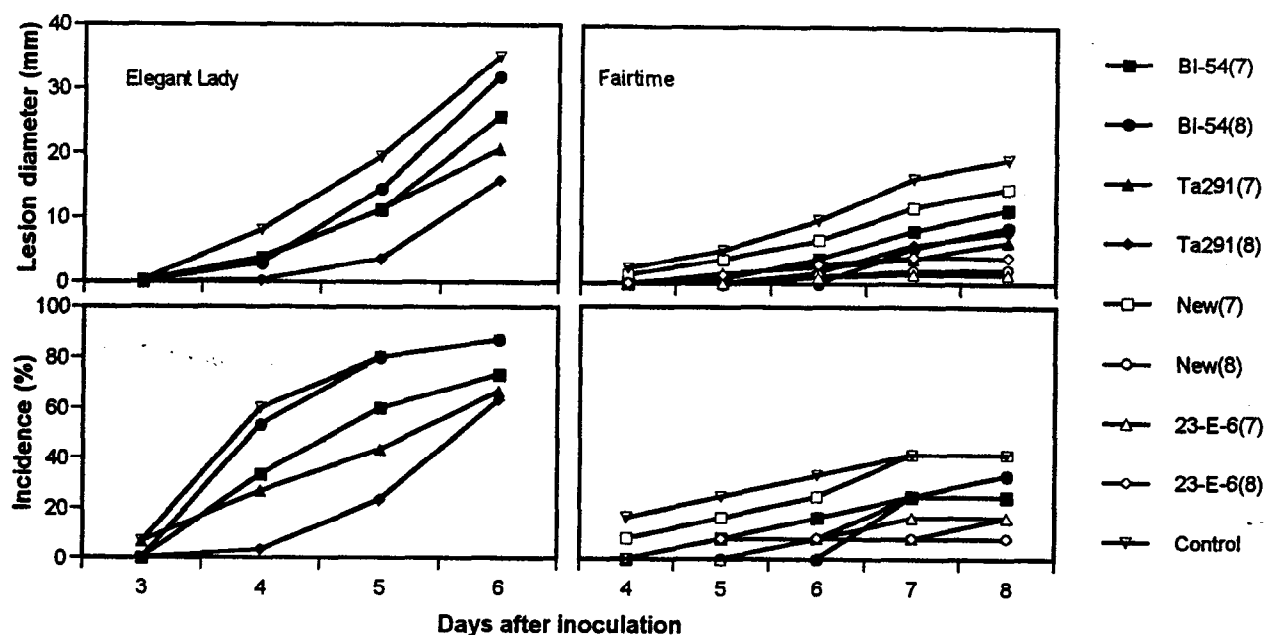
Figure 9. Brown rot development on unwounded peaches (cvs. Elegant Lady and Fairtime) inoculuated with 20 microliter of a suspension containing 1000 spores/mL of *Monilinia fructicola,* immediately following the application of 20 microliter of biological control agent, incubated at 20 C and 95% relative humidity (RH) for 24 h, then at 20 C and 50% RH for the remainder of the incubation period. The number in parentheses following the isolate name represents the concentration applied, e.g. BI-54 (7) denotes that BI-54 was applied at 10 million spores/mL.
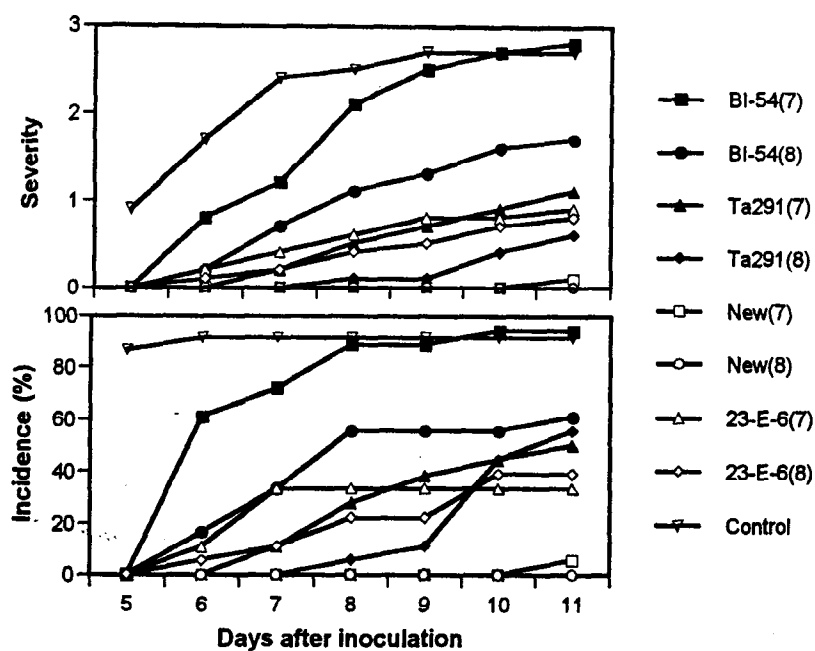
Figure 10. Brown rot development on wounded plums (cv. Casselman) inoculuated
with 20 microliter of a suspension containing 1000 spores/mL of *Monilinia
fructicola,* immediately following the application of 20 microliter of biological control
agent, incubated at 20 C and 95% relative humidity (RH) for 24 h, then at 20 C and
50% RH for the remainder of the incubation period. The number in parentheses
following the isolate name represents the concentration applied, e.g. BI-54 (7)
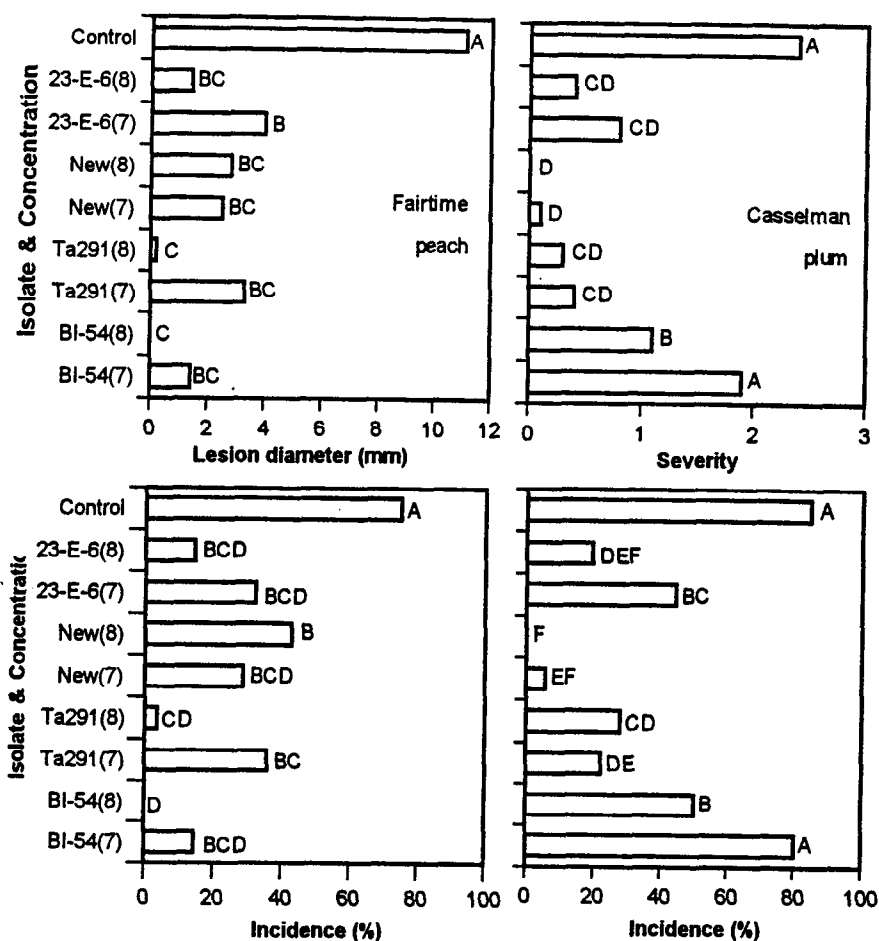denotes that BI-54 was applied at 10 million spores/mL.

Figure 11. Effect of biological control agent and inoculum concentration on the severity and incidence of brown rot of unwounded peach (cv. Fairtime) and wounded plum (cv. Casselman) fruit inoculated with 20 microliter of a suspension containing 1000 spores/mL of *Monilinia fructicola* immediately following the application of 20 microliter of each biological agent, incubated at 20 C and 95% relative humidity (RH) for 24 h, and then at 20 C and 50% RH for 3 and 6 days, respectively. The number in parentheses followed the name of isolates represents the concentration, e.g. BI-54 (7) denotes that BI-54 was applied at 10 million spores/mL. Mean bars topped with different letters differed significantly according to Fisher's least significance test at $P=0.01$.
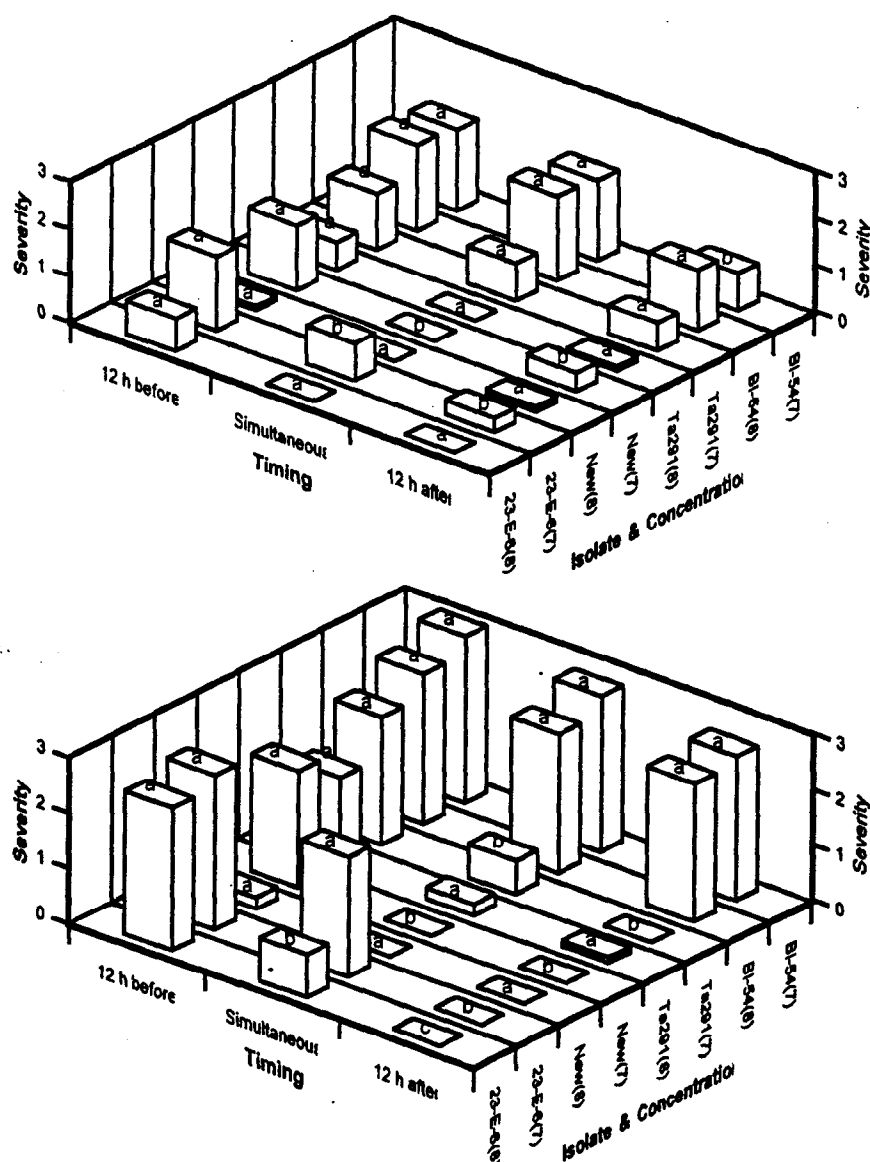
Figure 12. Effects of biological control agent and concentration on brown rot between wounded plums (cv. Casselman) fruit inoculated with 20 microliter of a suspension containing 1000 spores/mL (top) and 40000 spores/mL (bottom) of *Monilinia fructicola,* 12 h earlier, or 12 h later than the application of 20 microliter of the biological control agent or at the same time. Fruit were incubated at 20 C and 95% relative humidity (RH) for 24 h, then at 20 C and 50% RH for 6 days (top), and incubated at 20 C and 95% RH for 7 days (bottom). the number in parentheses followed the name of isolates represents the concentration, e.g. BI-54 (7) denotes that BI-54 was applied at 10 million spores/mL. Mean columns of the same isolate and concentration topped with different letters differed significantly according to Fisher's least significance test at *P*=0.05.
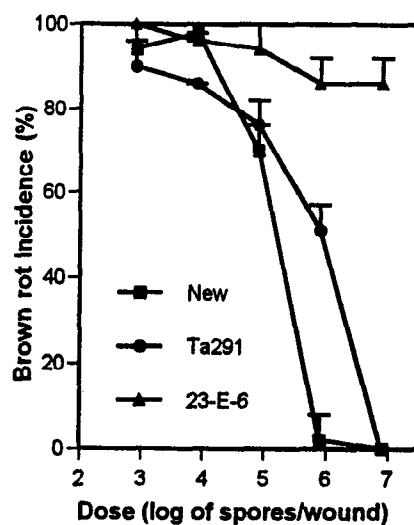
Figure 13. Influence of dose of biological control agent on brown rot incidence on wounded plum (cv. Casselman) fruit inoculated with 20 microliter of a suspension containing 80000 spores/mL of *Monilinia fructicola* immediately after the application of a biological control agent and incubated at 20 C and 95% relative humidity for 7 days.